II. REMARKS

Before the amendments made herein, claims 1 to 42 were pending. Claims 1 to 15 and 23 to 41 have been canceled herein without prejudice. In addition, claims 43 to 61 have been added. Accordingly, after the amendments made herein are entered, claims 16 to 22 and 42 to 61 will be pending.

A. Regarding the amendments

Claim 16 has been amended to more clearly indicate that the recited method is for determining the presence or absence of the recited compartment, component or macromolecule. The amendment is supported in the specification, for example, at page 17, lines 28-30.

Claims 16, 18 and 19 (as well as the new claims 43, 44 and 46 to 48) have also been amended to more clearly indicate that the recited method includes viruses. The amendment is supported in the specification, for example, at page 4, line 6.

Claim 18 has been amended to more clearly indicate that a nucleic acid construct encoding the chimer is provided. The amendment is supported in the specification, for example, at page 20, lines 23-30.

New claims 44 to 51 are directed to highlighting a cell compartment or biological component. The new claims are supported in the specification, for example, at page 3, line 23 to page 4, line 3.

New claims 52 to 59 are merely original claims 16 to 22, with the added changes discussed above. New claim 60 is supported in the specification, for example, at page 13, lines 8-9, and new claim 60 is supported in the specification, for example, at page 7, line 25 to page 8, line 2.

Because all of the amendments made herein are fully supported by the specification, no issue of new matter arises.

B. Regarding indefiniteness

Claims 16 to 22 and 42 are rejected as allegedly indefinite. Applicant respectfully traverses the rejection.

Claim 16

Regarding claim 16, it is alleged that it is not clear how one will provide a chimeric polypeptide to an organism or expose an organism to a detectable molecule.

Regarding providing a chimeric polypeptide to an organism, the specification discloses, for example, that "the chimeric polypeptide can either be administered (as a composition) to the organism, or alternatively and preferably it can be expressed within the organism as described above." See page 17, lines 8-11.

Regarding exposing an organism to a detectable molecule, the specification discloses that "Exposing the organism to the detectable molecule can be effected by injection, ingestion, or inhalation (in the case of a mammal), by providing the detectable molecule in the growth medium (in the case of cell cultures) or by irrigation, spraying and the like (in the case of plants)." See page 17, lines 12-15.

Claim 17

The Action alleges that it is not clear how a virus is an organism because organisms are self-reliant. While Applicant takes issue with the Action's allegation, to promote prosecution of this case, the claims have been amended to more clearly indicate they pertain to an organism and a virus.

Claim 18

The Action alleges that there is no antecedent basis for expressing the chimer of claim 16. In response, claim 18 has been amended to more clearly indicate providing a nucleic acid construct expressing the chimer.

Claim 42

The Action alleges that claim 42 is indefinite because it is not clear what the single chain Fv will bind or what the detectable molecule will be.

In response, this region of the chimer can bind a detectable molecule such as fluorescein. Specifically, as disclosed on page 21 of the specification: "A DNA fragment encoding scFv is isolated and cloned essentially as described by Lorimer *et al.* [PNAS 93: 14815-14820 (1996)] but with the exception of replacing the antigen used by Lorimer *et al* (a growth factor receptor) with the detectable molecule fluorescein.

Briefly, mice are immunized with fluorescein-conjugated keyhole limpet hemocyanin in complete Freund's adjuvant. Five days after the final immunization boost, spleens are harvested and poly(A)+ mRNA is purified therefrom using a Ficoll cushion and a FastTrack mRNA isolation kit (Invitrogen). The mRNA is utilized to construct a display library using the recombinant phage system from Pharmacia following the manufacturer's protocol. The final ligation reaction is used to transform *E. coli* TG1 cells (Stratagene) by electroporation. Phages are purified from the TG1 cell culture supernatant and suspended in a solution containing skim milk powder with Tween 20 in PBS containing biotinylated fluorescein. This suspension is mixed end-over-end at room temperature and then added to Dynabeads M-280 streptavidin-coated magnetic beads (Dynal, Great Neck, NY) that are blocked with a solution containing skim milk powder with Tween 20 in PBS. Following incubation, magnetic beads are captured with a magnet and washed with PBS. Beads are resuspended in PBS and used directly to re-infect log phase TG1 cells. The captured phage is analyzed for fluorescein specific scFv

by ELISA and the ELISA positive phage is used to prepare phagemids. Finally, the scFv DNA is PCR amplified from a selected phagemids."

For all the foregoing reasons, Applicant respectfully requests that this rejection be withdrawn.

C. Regarding enablement

Claims 16 to 22 and 42 are rejected as allegedly not enabled by the specification, citing various "Wands factors." Applicant respectfully traverses the rejection.

Factor 2

Among other things, referencing Wands factor 2, the Action alleges that "The specification does not state why one of ordinary skill in the art would want to target a cell compartment or biological component, or macromolecule in an organism."

In response, the specification discloses that the claimed invention "can be used to selectively stain or highlight cell compartments, biological components or macromolecules." See, for example, page 1, lines 9-10. Such selective staining is done, for example, to study these compartments, components and macromolecules. As disclosed in the specification, this indeed is an important funcition of many other technologies, including specifc dyes and reporter genes.

The present invention can also be used for diagnoses. "For example, by employing a chimeric polypeptide capable of binding a biological component or macromolecule associated with an abnormal phenotype, the present methodology can be utilized to detect cells exhibiting an abnormal phenotype (e.g., cancer cells). Alternatively, by employing a chimeric polypeptide capable of binding a viral component or macromolecule, the present methodology can be utilized to detect virally infected cells. It will be appreciated that in such diagnostic cases it is advantageous to compare

the resultant highlighted pattern of the tested cell or organism with that obtained from control cells (e.g., non-infected cells)." Specification, page 18, lines 1-9.

Factor 1

In referencing the first factor, the Action alleges that one skilled in the art would have to devise her own experiments to highlight a compartment, component or macromolecule endogenous to the organism. This, however, is not true. The specification provides an example of how to carry out the claimed invention, specifically by highlighting the mitochondrion of a mouse.

More specifically:

Preparation of a DNA fragment encoding fluorescein specific scFv:

A DNA fragment encoding scFv is isolated and cloned essentially as described by Lorimer *et al.* [PNAS 93: 14815-14820 (1996)] but with the exception of replacing the antigen used by Lorimer *et al* (a growth factor receptor) with the detectable molecule fluorescein.

Briefly, mice are immunized with fluorescein-conjugated keyhole limpet hemocyanin in complete Freund's adjuvant. Five days after the final immunization boost, spleens are harvested and poly(A)+ mRNA is purified therefrom using a Ficoll cushion and a FastTrack mRNA isolation kit (Invitrogen). The mRNA is utilized to construct a display library using the recombinant phage system from Pharmacia following the manufacturer's protocol. The final ligation reaction is used to transform *E. coli* TG1 cells (Stratagene) by electroporation. Phages are purified from the TG1 cell culture supernatant and suspended in a solution containing skim milk powder with Tween 20 in PBS containing biotinylated fluorescein. This suspension is mixed end-over-end at room temperature and then added to Dynabeads M-280 streptavidin-coated magnetic beads (Dynal, Great Neck, NY) that are blocked with a solution containing skim milk powder with Tween 20 in PBS. Following incubation, magnetic beads are captured with a magnet and washed with PBS. Beads are resuspended in PBS and used directly to re-

infect log phase TG1 cells. The captured phage is analyzed for fluorescein specific scFv by ELISA and the ELISA positive phage is used to prepare phagemids. Finally, the scFv DNA is PCR amplified from a selected phagemids.

Preparation of a DNA fragment encoding mitochondria binding peptide: The DNA fragment encoding mouse S-AKAP84 binding peptide is isolated and cloned essentially as described by Chen *et al.*, (1997) [J. Mol. Chem. 272:15247-15257]. Briefly, complementary DNA encoding human S-AKAP84 [Lin *et al.*, (1995) [J. Biol. Chem. 270, 27804-27811] is digested with *Nde*I and the resulting 1.8-kilobase pair fragment is used as a template to generate a random-primed, ^{32}P-labeled probe. This probe is used to screen a mouse testis cDNA library in bacteriophage λgt11 (Clonetech) Positive recombinant phage clones are plaque purified and cDNAs (0.9-2.9 kilobase pair) are subcloned in plasmids pGEM7Z (Promega) and pBluescript (Stratagene). A selected fragment of S-AKAP84 cDNA is amplified by PCR.

Generating the nucleic acid construct encoding the chimeric polypeptide: The DNA fragment encoding fluorescein-specific scFv is fused in frame to the DNA fragment encoding S-AKAP84. the fusion product is PCR amplified and the PCR product is cleaved with *Hind*III and *Xho*I and cloned into the expression plasmid pCEP4 (Invitrogen) which is cleaved with the same enzymes. This places the chimeric DNA downstream of the cytomegalovirus promoter and upstream of the polyadenylation signal. pCEP4 also contains a bacterial hygromycin B phosphotransferase gene under regulation of a strong viral thymidine kinase promoter. The isolated construct (Figure 1) is sequenced to verify sequence integrity. The polypeptide expressed from the nucleic acid construct is illustrated in Figure 2.

Transfection of cells with the nucleic acid construct, administration of the fluorophore and visualization: Human embryonic kidney cells (HEK293) are grown and transfected as described by Ndubuka *et al.*, (1993) [J. Biol. Chem. 268:7621-7624]. Transfected HEK293 cells are immersed in a fluorescein solution then thoroughly washed. Fluorescence signals and highlighted mitochondria are observed using a DeltaVision digital microscopy system (Applied Precision, USA). Specification, page 21, line 1 to page 22, line 25.

Moreover, in view of this guidance, the skilled artisan can carry out this procedure to highlight other targets, for example, a cell wall (page 12, line 29 to page 13, line 5), a low density lipoprotein or lipid (page 13, lines 10-16), a polynucloetide such as psbA mRNA (page 13, lines 17-22) or a polypeptide such as an MAP or an actin binding protein (page 13, line 23 to page 14, line 7).

Remaining factors

As to the remaining factors, the Action alleges that the art is complex and that the state of the art does not recognize the claimed methods. However, if this were the criteria, no invention would pass this factor. For by definition, an invention is novel and unobvious and, therefore, would not be recognized by the state of the art.

While the claimed methods are clearly novel and unobvious, the skilled artisan would know how to carry out each of the claimed **steps** because each step is known in the art. It is the **totality** of the steps wherein lies the claimed invention.

For example, making a chimeric is clearly known in the art. See for example, page 14, line 8 to page 16, line 22. Similarly, providing a chimeric to an organism is also known in the art. See for example, page 17, lines 8-11. In addition, exposing an organism to a detectable molecule is also known in the art. See for example, page 17, lines 12-15.

Thus, because each step of the invention is known in the art the nature of the invention is well within the level of the skilled artisan to perform.

Finally, Applicant notes that the Action alleges that the claims are broad in rejecting each and every claim under examination, no matter how narrow.

For all the foregoing reasons, Applicant respectfully requests that this rejection be withdrawn.

D. Regarding anticipation

Claims 16 to 22 are rejected as allegedly anticpated by Xu et al. Applicant respectfully traverses the rejection.

The Action alleges that Xu teaches a method of highlighting a macromolecule (KaiB) in an organism by providing the organism with a chimer and exposing the organism to a detectable molecule.

In response, Applicant points out that highlighting the macromolecule KaiB is not the point of Xu. Rather, its point is to study the macromolecule's interaction, specifically with itself.

More importantly, merely highlighting the macromolecule in Xu is done by the binding of the detectable molecule to the construct, a construct that was made to include the macromolecule itself. To merely highlight the macromolecule, there is no need for Xu to have a second polypetide region being capable of binding the macromolecule, as is required by the subject claims. Thus, the point of Xu is not to detect the presence or absence of the macromolecule, which is obviously already known.

Therefore, to more clearly distinguish the claimed invention from Xu, Applicant has amended claim 16 (as well as all claims dependent thereon) to require that the exposure of the organism or virus to the detectable molecule be to determine the absence or presence of the cell compartment, biological component or macromolecule. By contrast, the method of Xu is not at all for such purpose.

New claims 52 to 59 actually retain the original scope of claims 16 to 22. They are distinguished from Xu for reciting the phrase "a second polypeptide region capable of specifically binding the . . . macromolecule **of** the organism" in step (a)(ii). This means that the recited macromolecule originated in the organism as is therfore "of" the

organsim. The top of page 4 of the Action itself recognizes this point by characterizing the claimed invention as highlighting a macromolecule **"endogenous"** to an organism.

By contrast, the macromolecule of Xu is not endogenous to the organism at issue. Specifically, Xu studied proteins from cyanobacteria by expressing them in *E. coli*. As discussed above, the point of Xu was to study the proteins' interaction, not to identify the presence or absence of the protein that was genetically engineered into the organism in the first place.

To emphasize this point even further, new claim 60 is directed to where the macromolecule is cell specific. This is obviously an endogenous concept. By contrast, the macromolecule of Xu is not only non-cell specific, it is foreign to the cell.

Similarly, new claim 61 is directed to localizing the detectable molecule to a specific area. By contrast, Xu does not do this and is simply interested in studying its protein of interest anywhere in the *E. coli* cell.

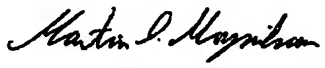
Finally, new claims 43 to 51 are directed to highlighting a cell compartment or biological component. By contrast, Xu does not teach or suggest this.

For all the foregoing reasons, Applicant respectfully requests that this rejection be withdrawn.

III. CONCLUSION

All of the issues raised in the Office Action have been addressed and are believed to have been overcome. Accordingly, it is respectfully submitted that all the claims under examination in the subject application are allowable. Therefore Applicant respectfully requests a Notice of Allowance to this effect.

Respectfully submitted,



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Date: July 23, 2007

Enclosurs:

- Petition For Extension of Time (Two months)
- Additional Claim Fee Transmittal